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14. ABSTRACT This study focuses on the role of inflammatory complement proteins, which are elevated during a prolonged asymptomatic period in mild repeated traumatic brain injury (TBI), in chronic traumatic encephalopathy (CTE). We propose there is synergism between these proteins and aberrant tau aggregates, a common downstream pathway in many neurodegenerative disease. A central goal of this study is to create and characterize two novel bigenic mouse models that will define the interaction between human tau and these inflammatory proteins, using a repeat lateral mild, head trauma model to recapitulate common injuries suffered in combat or sports. During this **third year** we have produced approximately 80% of the required mice, overcoming obstacles with rederivation, breeding and low yield of bigenics. We have performed most of the TBI's on these mice and are currently analyzing the single transgenic mouse brains both biochemically and histologically. We have identified TBI- and transgene-dependent locomotor disturbances associated with neuroinflammatory pathology. Further we have made progress in the development of GFAP and ER stress markers as brain-derived plasma biomarkers that will be helpful in dissecting the dynamics of neuroinflammation in the complex prodromal sequalae of CTE. Effects of mTBI on tissue contralateral to the injury is also identified, such as increased soluble and insoluble tau aggregates of tau and glia.

15. SUBJECT TERMS Glia, microglia, mild traumatic brain Injury, chronic traumatic encephalopathy, complement cascade, neuroinflammation, neurofibrillary tangles, tau, trans-synaptic, phagocytosis

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1. **INTRODUCTION:** The purpose of this study is to use animal models to elucidate the mechanisms after repeated mild traumatic brain injury (mTBI), leading to neurodegenerative disease, such as chronic traumatic encephalopathy that occur after several asymptomatic months or years. This long asymptomatic period suggests that the brain has strong protective mechanisms against deleterious effects, but that eventually there is failure to compensate. The main pathology thought to cause onset of the disease is accumulation of abnormal aggregates of a protein called tau, which is a pathology common to many neurodegenerative diseases. Chronic aberrant neuroinflammation (dysregulation of astrocytes and microglia), during the asymptomatic period is known to drive tau pathogenesis through activating tau kinases, but the mechanisms remain elusive. We have identified an inflammatory pathway called the complement cascade involving the microglia, which plays an essential role in synaptic pruning, but no model to date has modeled its hyperactivation, known to occur after TBI. Since our preliminary data shows that C1q plays a prominent role in tau accumulation and that these effects are mediated by C5 convertase, we have obtained novel models that will for the first time allow study of these mechanisms. Our data also show that the complement cascade plays a role in tau accumulation that is distinctive and opposite from its role in amyloid accumulation. This proposal investigates the hypothesis that the dysregulation of glia plays a critical role in tau spreading leading to cognitive loss.

2. **KEYWORDS:** Microglia, Astrocytes, tau, complement 5a, serpin, C1 esterase inhibitor, tau kinases, chronic traumatic encephalopathy, post-traumatic brain injury, trans-synaptic, phagocytosis.

3. **ACCOMPLISHMENTS:**
   - **What were the major goals of the project during this Period (Year 3)?**

     The obstacles described in the first year delayed completion of the aims in this three year project, leading to a revised SOW to extend this project to Year 4. This Year 3 report is based on the original SOW,

     Major Task 3 (Subtasks 3.2-3.7): To complete the animal portion of the experiments (Sham vs repeat mild TBI) with the four C5a groups to evaluate whether C5a, a byproduct of complement activation creates a chronic aberrant inflammatory response contributing to the prodromal phase preceding delayed behavioral impairment associated with glial dysfunction that accelerates tau pathology.

     Subtasks 3.2 (Subject C5a groups (C5a, C57, htau, C5a/htau). to mTBI or sham and age them out), 3.3 (subject them to behavioral testing), 3.4 (Euthanize and collect blood and dissect brains for evaluating biochemical indices of pathogenesis), 3.5 (Collect synaptosome collection), 3.6 (fix tissue for histology) and 3.7 (EM).

     Major Task 4 (Subtasks 4.1-4.5): To perform analysis of tissue samples and behavioral data for above C5a study.

     Subtask 4.1 (Behavioral data analysis), 4.2 (Regional accumulation tau in different biochemical fractions/compartments), 4.3 (Biochemical variables of neurodegeneration) 4.4 (Present findings at conference), 4.5 (manuscript).

Major Task 5: Breeding the hu Tau Tg and huC1q and aging them out.

Major Task 6 (Subtasks 6.3-6.8): To complete experiments on C1q Tau Tg with the four groups to assess whether releasing the brake on this first step in complement, perpetuates a chronic inflammation and binds to tau and accelerates tau deposition.

Subtask 6.3 (mTBI), Subtask 6.4 (Perform Behavior), 6.5 (Euthanasia and Collection of tissue), 6.6 (synaptosomal), 6.7 (Confocal and ICC microscopy for senescent GFAP) –6.8: Collect tissue for Electron microscopy of glial-synaptic and glial-tau associations.

Major Task 7: Data Analysis C1q/tau

7. 2 (Analyze behavior), 7.3 (tau accumulation) 7. 4 (biochemistry) 7.5 conference (7.6-manuscript)

Major Tasks 8-9 (Subtasks 8.1-8.3 and 9.1-9.5): Determine glial role in tau accumulation related to C5 overexpression (Task 83) and C1q (Task 9).

8.1 / 9.1: (Synaptosome Examine glial-synaptic associations in vulnerable regions, both models). 8.2 /9.2: (ICC microscopy for senescent GFAP proteins and glial –specific antigens implicated in tau uptake or toxicity, both models)
8.3 / 9.3 (Electron microscopy of glial-synaptic and glial-tau associations).
9.4 (conference) and 9.5. (Prepare and submit manuscript for publication).

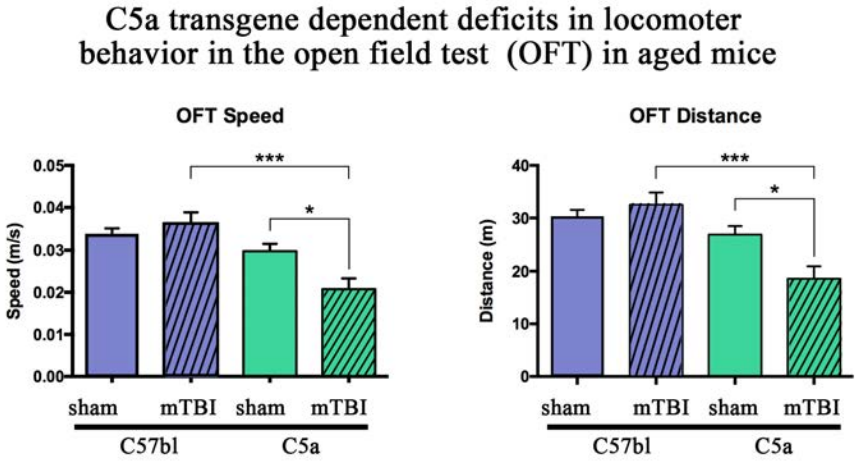
- **What was accomplished under these goals?**

Accomplishment 1: All the monogenic and wildtype mice have been aged out and euthanized and undergone behavioral testing. Producing the bigenics has been problematic as explained in past reports, but we are now have created both models and are producing enough to complete this study in a fourth year. It is critical to get these data as understanding tau-synergism with this specific type of inflammation will fill a major knowledge gap. We have produced 75% of the bigenic offspring needed and subjected about 50% of these subjected to mTBI which are currently being aged out. The remaining bigenics will undergo mTBI in the first and second quarter of the 4th years.

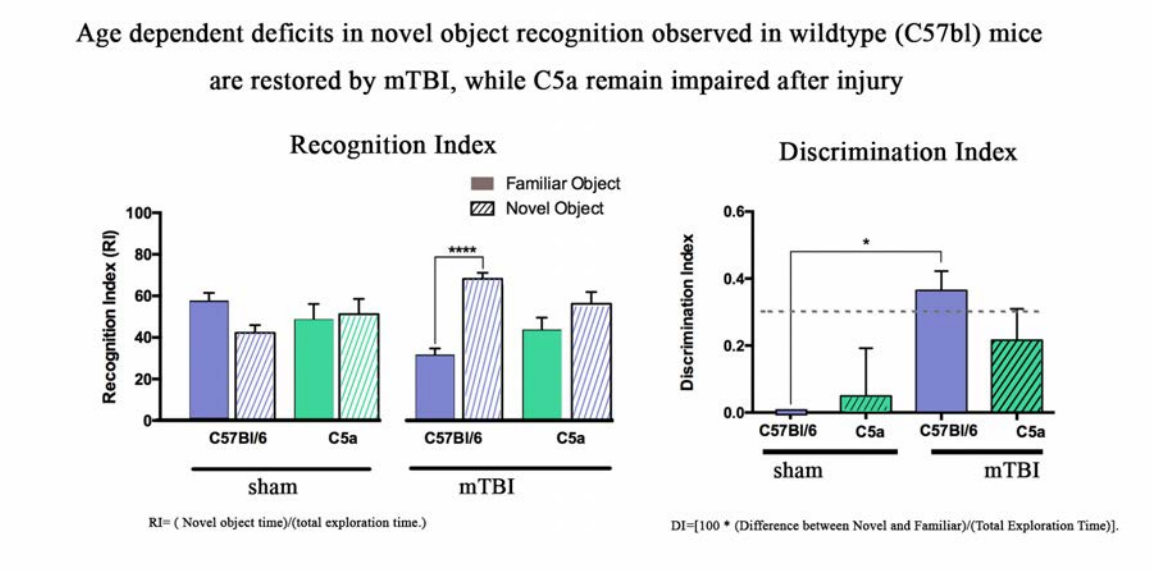
Accomplishments 2: We have presented the work at a TBI conference in Tampa at University of South Florida (May 17, 2018, see section on dissemination to the community).

Accomplishment 3. We have written the introduction to the C5a paper (appended to this progress report).

Accomplishment 4: 4a: We have analyzed the behavior for the groups that have completed the experiments. Despite not having produced the key tau groups (due to backcrossing, yield an rederivation previously described) we have accumulated data on the other completed groups, which will allow us to submit a paper in the second quarter of the fourth year, prior to completely the bigenic portion.



4b: In this study we had a large cohort of aged mice (16-18 months) which had been aged out because of the number of mice needed for breeding. This gave us an opportunity to assess the effect of age and transgene. Open field performance testing showed deficits in these aged C5a mice, compared to control C57bl after mild repeat TBI in aged mice. Speed and distance were significantly reduced in C5a-GFAP mice that received rmTBI, compared to sham C5a ($p<0.05$) and injured C57bl ($p<0.001$). In C57bl mice, the speed and distance were the same, regardless of whether they were subjected to injury. These data suggests that overexpression of C5a in the CNS increased susceptibility to locomotor dysfunction as a result of rmTBI. Since these mice are ambulatory, and have no motor deficits, it appears the effects may relate to lack of motivation and depression and model the post-concussive syndrome, typical of patients with mild TBI where the symptoms outlast the recovery by years. Clinical research with patients with mild traumatic brain injury suggests a high prevalence of sustained or delayed neuropsychological, and emotional disturbances, including depression While only 15% of subjects develop major depression (1), a post-concussive syndrome with mild depression frequently outlasts the recovery for years (2), which is supported by metanalysis (3). Severe fatigue as an early response to injury increases the likelihood of long term post-concussive symptoms (4). In our experiment, this cohort of mice was aged up to 16 - 17 months; aging is a known risk factor for predicting this decline (5). Although it is not economical or practical to conduct research in animals this old, these data provide important knowledge in the field of CTE, which is still at an early stage, where the etiology remains elusive.



4c. We also tested cognition (Barnes Maze and Novel Object Recognition). Not surprising, was that in these aged mice, neither the C57bl or C5a mice could differentiate between novel and object, showing equivalent age-related impairment. What was surprising was the transgene differences in the response to injury, om that the C57bl injured mice regained their ability to distinguish between novel and familiar as calculated by the recognition index (RI). This may be due to the known initial increases in growth factors and other repair mechanisms after injury. Relatie to the C57bl, the C5a retained deficits. It could still not differentiate between novel and familiar. Thus the wildtype mice developed a significant preference for the novel object in the test phase, suggesting intact working memory, while the C5a remained impaired. In this paradigm, there are two important outcomes. First it was surprising to see that the response to injury improved memory, however this phenomenon has been described in the literature (6). Although there is acknowledgment that the sequelae between TBI and onset of disease is complex and to move the field forward there will have to be a 'paradigm shift' (7). Currently in preclinical research, there is an attempt to rush to model the delayed effects of injury, without appreciating dissecting out the multiple prodromal phases, which may give more insight into the causes of CTE. Here data in the wildtype mice captures a prodromal stage of cognitive improvement, which may disguise a pathological trigger as evidenced by the pathology in these mice. The identification of brain-derived blood biomarkers that are not just useful diagnostics but can better characterize progression is likely to be one methodology to elucidate the complex sequelae. Second, unlike in wildtype, the age-related deficits in sham-treated C5a were not restored by injury. This experiment was done with a 6 week delay between the mTBI and behavioral testing as opposed to the other groups, so would be more likely to detect an earlier step in the pathogenesis. These data suggest that overexpression of C5a in the CNS prevents the injury-induced restoration of NOR memory we saw in aged C57 mice.

4d. Synopsis of other behavioral data from year 3 quarterly reports:

4d.i. (Middle aged groups): Y Maze: We obtained Y maze data in the htau and Serpin KO: reported in Year 3 quarter 1. The cohorts were middle aged mice with 3 months post mTBI assessment as opposed to the aged mice with 6 weeks post mTBI assessed above. In Y maze, compared to C57bl, the htau show reduced

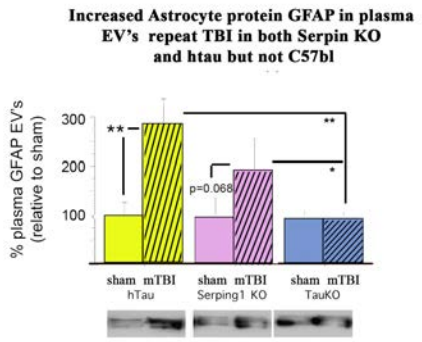
activity in response to injury in the Y maze without manifestation of cognitive deficits. However, there was a tendency for reduced arm entries, distance traveled and speed in the htau, C5a mice, arguing that, although cognition may not necessarily initially be affected by repeat mild TBI, there is a general apathy or depression. Barnes Maze studies of acquisition and other testing in these cohorts confirmed that the cognition and motor function were intact but htau mice subjected to injury would not explore. The hypoactivity associated with TBI was not seen in wildtype mice subjected to injury nor in the Serp1 KO. This demonstrates that tau accumulation does impact long term outcomes after mTBI.

4d.ii. NOR (middle aged). The mTBI associated reduced locomoter activity in specific to the htau mice was also observed during the Novel Object recognition test.

Collectively, these behavioral data analyzed in year 3, suggest that there is a reduction in locomotor activity in the htau transgenic mice (htau middle aged, 3 month post-TBI period) and C5a (aged, with shorter post-mTBI period) but not in the Serpin KO. This supports that both tau and the inflammatory component C5 can contribute to a post-concussive – like syndrome.

Accomplishment 5.

We have also made progress in assessment of brain derived blood biomarkers.

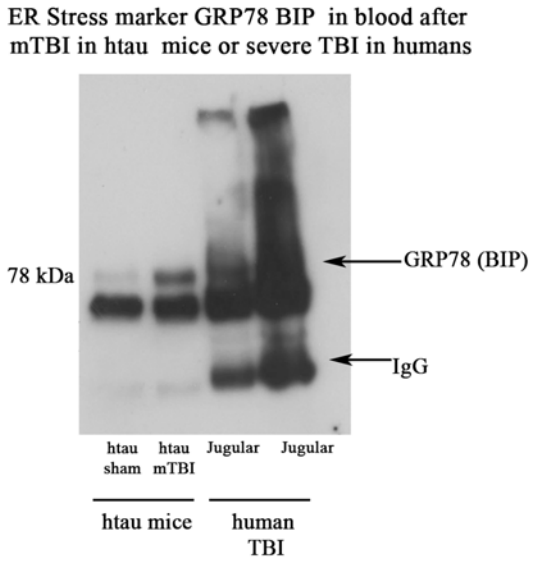


5.a In this project we have shown the astrocyte end feet protein aquaporin-IV as a viable blood biomarkers to track progression and understand sequalae (prior reports).

5b. We have more data supporting GFAP in EV's as a predictive biomarker.

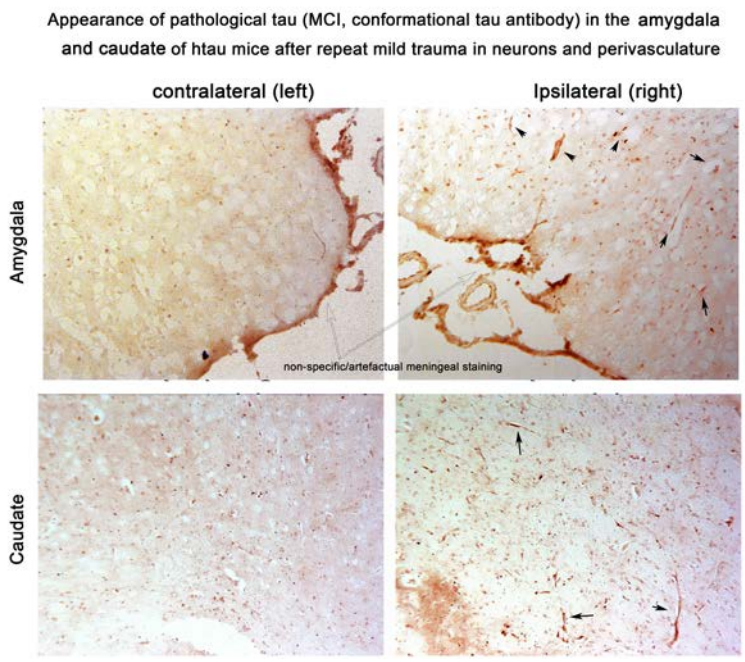
This figure demonstrates that the increased GFAP in most proiminent in the htau mice there also appears to be a trend for increase (p=0.068) in the serpin while GFAP in the sham C57bl controls is not elevated compared to mTBI. Data was analyzed by ANOVA (brain trauma: TBI or sham) and Tg (htau, tau KO or serpin. TBI responses relative to sham were normalized to sham for the respective transgene. Log transformation was necessary to establish homogeneity. F value for the corrected model was significant, showing treatment differences. $F_{(4,31)}=3.820$, $p=0.01$. The main effects for trauma was also significant $F_{(1,31)}=8.645$, $p=0.001$. Post hoc LSD tests showed differences between sham and mTBI in the htau, non-significant trend in serpin and no effect in C57bl.

5c. We are also exploring an ER stress marker in plasma BIP (GRP78). As a positive control, we use human tissue from subjects recovering from severe brain injury in which arterial and jugular blood are collected. This allows comparison of blood enriched in brain proteins (clearing the brain and returning to the hear via the jugular). Our htau mice with mTBI show increased levels of BIP and, in human TBI, the levels are higher in the jugular than the artery, supporting a brain origin. We are now going to assess this in a larger sample group.

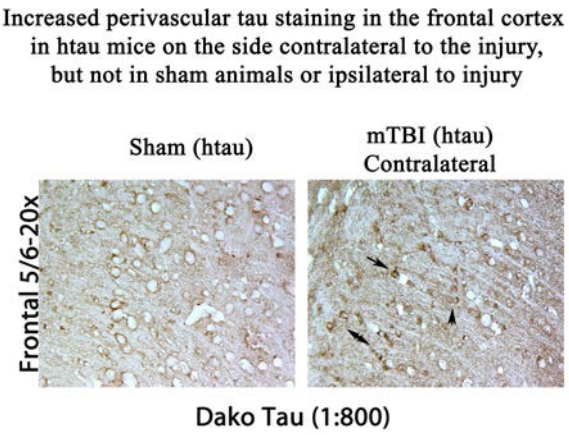


Accomplishment 6.

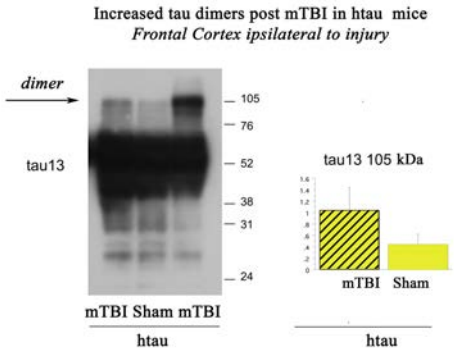
6.1 A major goal in this project is to track changes in tau and understand why it accumulates perivascularly in CTE. Initial data show some evidence of pathological tau (MCI) which is an antibody to a pathological conformation of tau. This is mos prominent in the amygdala and caudate.



6.2 We also stained tissue for total tau using DAKO antibody (binds all forms of tau, both phosphorylated and dephosphorylated.) Measuring tau in the mouse brain has proven difficult (8). Some of this is because the assays are mouse antibody (to pathology) on mouse tissue. We use tau KO model as a negative control to assess specificity, although AT8 can label other MAPs with homology to tau. What helped was to dilute the antibody 4 fold more than used for our positive control human tissue, which greatly increases the specificity. This panel shows more tau on the contralateral side to injury, perhaps due to the lateral movement of the brain against the bone of the contralateral side. This emphasizes the importance of comparing left and right with injury and with sham.



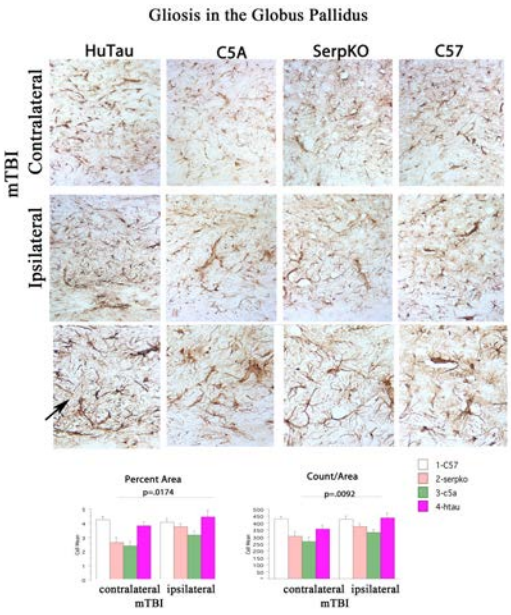
Accomplishment 7.



We also assessed tau in Western fractions. On the ipsilateral side, we observed increased tau dimers in the cortex in response to mTBI, a known pathological aggregate contributing to cognitive decline in Alzheimer's. This blot shows tissue from htau mice with or without mTBI in a soluble fraction using an antibody specific to human tau. The monomeric form shows a very strong band, and even with shorter exposure, there is no difference in that band. However the dimer is increased in response to mTBI.

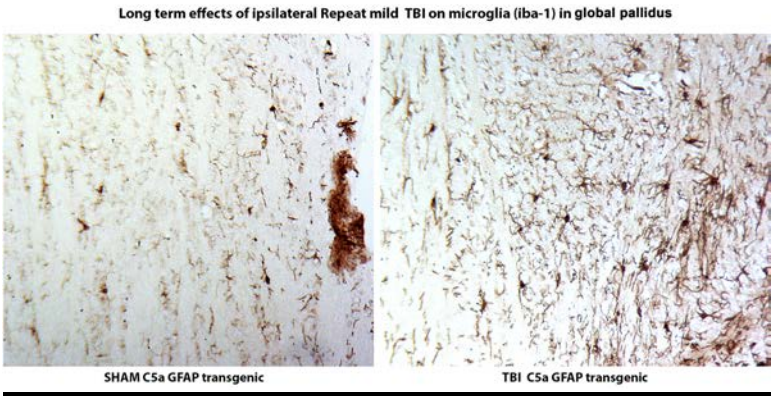
Accomplishment 8:

8.1 We identified what appeared to be a vulnerability of the globus pallildus, in terms of astrocytosis and gliosis. Dr. McKee, who first characterized this disease, has described the globus pallidus as one of the vulnerable regions to perivascular tau, perhaps because of its denser vasculature. (9). We characterized the morphology of the changes in detail, and the results were as follows: Transgene effects were identified in all morphological variables assessed with the htau>C5q>serpin, including the: shape of the astrocyte processes (which were longer and more swollen in the htau mice after mTBI), (the % area, the intensity of staining, cell size and number of cells. The astrocytes in the ipsilateral side to injury in the htau had very different morphology. There was a denser network of fibers, which appeared thinner than in other groups, and the cell bodies were darker and appeared pyknotic. In all the transgene the injury side showed significantly more staining than the contralateral side. The increased density of the more extensive network of processes in the TBI groups did not appear to be picked up as the visual differences were more marked than the quantify data, so we are going to rewrite the macro and analyze at higher magnification. GFAP levels were high in wildtype, leading us to analyze sham C57bl vs trauma, and we confirmed the minimal ipsilateral vs contralateral differences, and that GFAP was increased in response to injury in the wildtype. The morphology of the astrocyte in the transgenic is very different than that of the C57bl, but this raises the question as whether there is senescence in these transgenes in response to injury or whether this is an artefact of our image analysis which failed at that magnification to pick up the smaller processes. We will resolve this problem by staining with antibodies to senescent astrocytic proteins. Distinguishing between 'healthy' and pathological astrocytes is an important goal in this project.



Two way Anova GFAP			
	treatment	ipsi/contra	interaction
Process length	f(3,63)= 25.6 , p <0.002	F(1,65)=6.014, p =0.001	NS
Count/area	f(3,63)= 8.7 , p <0.002	F(1,65)=7.2, p =0.009	NS
cell size	f(3,63)= 3.73 , p <0.002	NS	F(3,65)=3.55, p=0.0192
percent area	f(3,63)= 19.3 , p <0.01	F(1,65)=4.014, p =0.01	NS
intensity	f(3,63)= 15.1 , p <0.0001	F(1,65)=10.2 p =0.002	F(1,65)=5.329, p =0.0024

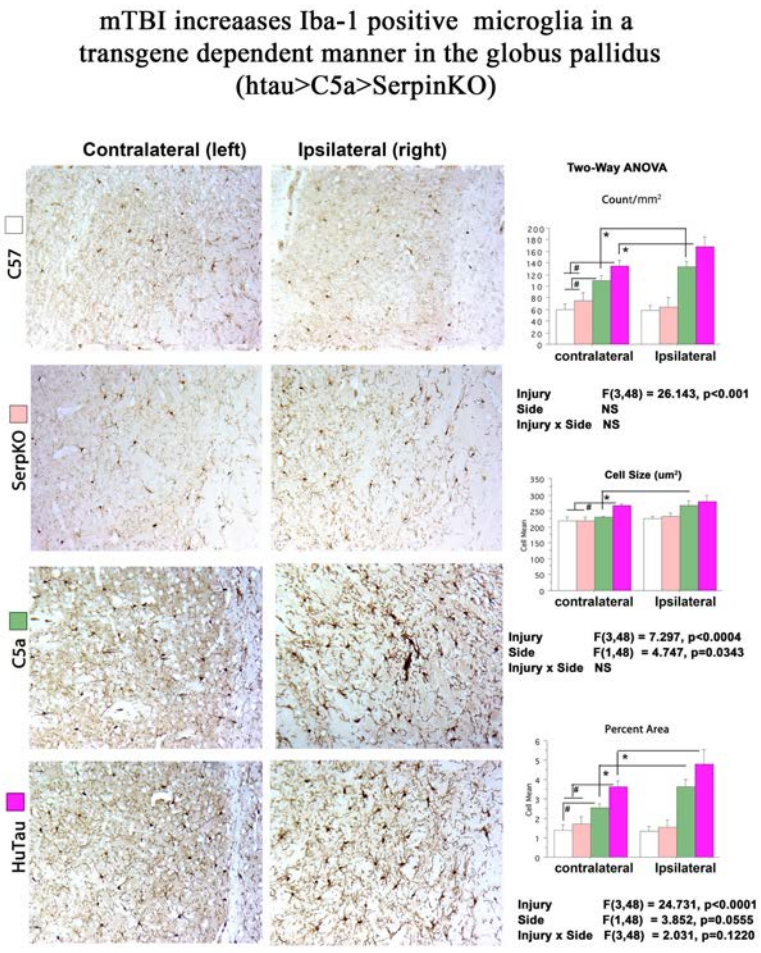
8.2 This higher magnification demonstrates the robust effect of the C5 transgene on inducing gliosis in the globus pallidus.



8.3

Iba-1 changes in the globus pallidus paralleled the transgene dependent changes in GFAP

In this panel, we show ipsilateral contralateral differences but have not compared sham to injury, which is important as we now know that both sides are affected in this injury.



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What opportunities for training and professional development has the project provided?

- We have three undergraduate students receiving course credit to perform research in this program during the year. They are learning to perform behavior, download the data, analyze it statistically, present their work in writing, including conducting background literature search as well as in laboratory meetings. They are learning Western blot to analyze biochemical changes and assisting in sectioning brains and immunostaining for pathological protein changes. They include Carmen Leal, Marisa Mekkittikul and Danielle Tran. Danielle Tran left for medical school at UCSD, but Carmen and Marisa will be working on this project this year. We also have admitted Farrah Au-Yeung who has not been trained yet.
- In addition to undergraduate students we have two skilled visiting scholars who are neuroscientists skilled in neurosurgery and behavior: Dr. Cansheng Zhu and

Dr. Katsuya Sakimura. They have their own projects, but are assisting in euthanasia, tissue collection and biomarker studies, as the assessment of biomarkers and establishment of new methods on their projects in AD (ApoE4, PS1/APP, human AD) will be beneficial to our assessment in TBI.

- Finally two post-graduate students who were previously on the SRP199 course program: Trevor Nguyen and Nisha Choothakan, worked on Western blot analysis and biochemical processing of tissue. They have also left to go to medical school (both UC San Francisco).

- **How were the results disseminated to communities of interest?**

I presented the data in a lecture entitled "Role of complement proteins after TBI on tau accumulation" (May 17-18, 2018) at the a TBI conference at University of South Florida, which was organized via the Veteran's administration (Dr. Mark Kindy). The conference was entitled "New perspectives on central and peripheral inflammation in traumatic brain injury: Program to study the impact of inflammatory state in TBI in the VA population and establish collaborative research programs". The purpose was two-fold: first to bring together major laboratories researching brain pathology from postmortem tissue of people with head trauma (Ann McKee) and different models of TBI (blast injury) , CTE (Fiona Crawford), blast injury (Dr. Zezong Gu) and several other laboratories, all affiliated with the VA and the associated universities to discuss where the field is and where it has to go. The second purpose was to plan collaborations that will result in funding to extend our current projects and accelerate advancements in the field. As a result we have worked together to produce and submit, a program with four linked VA projects with Dr. Ann McKee, Weimin Xia, Karen Ashe and Zezong Gu. Our group (Cole PI ,Frautschy CoPI) is responsible for CTE model and biomarkers for all groups, Dr. Ashe is providing a new tau transgenic mouse model that will be used by all groups, and Dr. Gu for the blast injury model. the title of our program and project is "BX004332 CTBI: Tauopathy in mice and human: Surrogate Plasma Biomarkers for Brain Trauma-Initiated Neurodegenerative Disease", and the data in this DOD project accumulated has been important for this proposal as well as Dr. Cole's biomarker data in TBI human subjects. During this year the proposal has gone through one review and we have already submitted a revised stronger program and addressed concerns and will hear the outcome of the resubmission shortly.

- **What do you plan to do during the next reporting period to accomplish the goals?**
  - *We have revised the Statement of work to accomplish the goals in the next period. We will place a large focus on the analysis of the single transgenic studies so that we can submit a paper on that data . This will include a more thorough assessment of senescent gilal proteins and their association with the vessel as well as full characterization of tau changes by ICC and Western..*

4. **IMPACT:**
   - **What was the impact on the development of the principal discipline(s) of the project?**
     - The impact on the principle discipline is unknown. The role of these proteins known to be present in humans after TBI is unproven and poorly studied at the basic level. The lack of the field's focus on investigating the complement mechanisms in animal models, despite these complement proteins showing up in nanostring and RNA seq data in human TBI is surprising..

Our data supporting a role in C5a exacerbating pathology in a CTE-like model, suggesting that this study is likely to have an important impact .

- **What was the impact on other disciplines?**
  - *This study may have an impact on understanding mechanisms of inflammation in other tauopathies (FTD, or Alzheimer's), particularly in overlap on biomarkers and role of glia. It may also intersect with mixed dementia risk in cardiovascular diseas, as it will allow investigation if this milieu contributes to vulnerability to poor outcomes in CTE and also explain the mechanisms of accumulation of vascular tau.*

  - **What was the impact on technology transfer?**

  - *This study may identify new biomarkers (AQP4, BIP GPR78 and GFAP) for TBI, We are working on developing a kit to detect neuroinflammation in plasma samples using the biomarkers identified in this project.*

- **What was the impact on society beyond science and technology?**
  NOTHING TO REPORT
- *T*

5. **CHANGES/PROBLEMS:**
   - **Changes in approach and reasons for change**. *NOTHING TO REPORT*
   - **Actual or anticipated problems or delays and actions or plans to resolve them**
   - *The main problem is the one year delay in the study The project is going smoothly.*
   - **Changes that had a significant impact on expenditures.** We have had to secure supplemental funding (unrestricted donor funds) to complete this project due to the high cost of salaried employees and animal per diem.
   - **Significant changes in biohazards or select agents. N/A/**
   - **Significant changes in use or care of human subjects. N/A**
   - **Significant changes in use or care of vertebrate animals. NO**
   - **Significant changes in use of biohazards and/or select agents. N/A**
6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*
   - **Publications, conference papers, and presentations** *Presented this work at the University of South Florida Conference on TBI. May 17-18, 2018*
   - **Journal publications.** *NONE*
   - **Books or other non-periodical, one-time publications.** *NONE*
   - **Other publications, conference papers, and presentations.** *NONE*
   - **Website(s) or other Internet site(s)** *NONE*
   - **Technologies or techniques**
     We are developing techniques to assess Plasma Extracellular Vesicles derived from the brain that may monitor inflammation related to TBI. Currently we are using human samples from another grant, and we can apply this new technology to the mouse models in this study.

   Inventions, patent applications, and/or licenses *NONE*

   Other Products N/A

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	*Peter Kim*
Project Role:	*Senior Research Associate 2*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*12*
Contribution to Project:	*Mr. Kim manages the colony and breeding and works with the PI to conduct the TBI. He genotypes mice and ensures that the appropriate number are bred for the DOD project and communicates weekly about progress. He is also responsible for overseeing the work of undergraduate students.*
Funding Support:	*N/A*

Name:	*Paul Denver*
Project Role:	*Post Doctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*12*
Contribution to Project:	*Dr. Denver is involved in participating in all aspects of analysis and working with the PI to supervise the completion of the studies and writing of the papers..*
Funding Support:	*N/A*

Name:	*Carmen Leal*
Project Role:	*Senior Research Associate 1*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*4*
Contribution to Project:	*Ms. Leal is assisting in behavioral analysis and animal care, and receiving academic credit for this research.*
Funding Support:	*N/A*

Name:	*Mychica Jones*
Project Role:	*SRA3*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*3*
Contribution to Project:	*Ms.Jones is the laboratory manager and histologist. She performs histology and image analysis and trains and supervises students in histology/*
Funding Support:	*N/A*

Name:	*Marisa Mekkittikul*
Project Role:	*Undergraduate student*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*1.64 months (no cost)*
Contribution to Project:	*Ms. Mekkittikul is working on the project caring for the mice and receiving academic credit. She will also be assisting with histology.*
Funding Support:	*N/A*
Name:	*Andrea Tenner*
Project Role:	*Director of MIND institute UC Irvine*
Researcher Identifier (e.g. ORCID ID):	*andreatenner*
Nearest person month worked:	*1 (no cost)*
Contribution to Project:	*Provided C5a Tg mice and assisting in recovering embryos and troubleshooting rederivation of the colony at UCLA*
Funding Support:	*T32 AG000096 "Training in the Neurobiology of Aging"* NIH NIA (PI, C.W. Cotman, Project Leader - A.J. Tenner) 5-01-14 through 4-30-19 $250,000 P01 AG 00538 "Behavioral and Neural Plasticity in the Aged", Project Neuroprotection and neuroinflammation induced by complement proteins and receptors $800,000 5-01-14 through 4-30-19

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  - *No*
- **What other organizations were involved as partners?**

        - *N/A*
    - **Personnel exchanges**
        - *N/A*

8. **SPECIAL REPORTING REQUIREMENTS**
    - **COLLABORATIVE AWARDS:** *N/A*
    - **QUAD CHARTS:** *N/A*
9. **APPENDICES:**
    - *Introduction to C5a Manuscript..*

Exacerbation of pathology and non-cognitive behavioral deficits in aged to C5a mice 4 months after repeat mild TBI. Denver, P. Jones, M., Kim, P.H., Teter, B. Hu, S., Frautschy, S.A.

Upon upstream activation of the alternative complement cascade, C5 convertase cleaves a C5 molecule into the anaphylatoxin C5a and bioactive fragment C5b (Merle et al., 2015). C5b interacts with several further complement components, culminating with the formation of the membrane attack complex C5b-9 (MAC), which induces membranous pore formation and lysis of the target cell (Merle et al., 2015, Hammad et al., 2018). It is understood that MAC formation exacerbates neuropathology and neurological dysfunction following traumatic brain injury (TBI) (Fluiter et al., 2014), implicating C5b as a critical down-stream component of the cascade that leads to MAC formation subsequent to TBI (Merle et al., 2015, Hammad et al., 2018). The role of C5a in the brain following injury however is less well characterized.

The C5a receptor (CD88) is expressed at low levels in normal brain, but is greatly up-regulated upon brain injury, with elevated expression detected in astrocytes, microglia and, to a lesser extent, endothelial cells (Gasque et al., 1997). C5a is involved in recruitment of neutrophils to the site of traumatic brain cryoinjury and exacerbation of injury size (Sewell et al., 2004), possibly via Ras/Raf/MAPK signaling (Buhl et al., 1994). Moreover, suppression of the alternate complement cascade and reduction of serum C5a has been found to attenuate neuropathology following TBI (Leinhase et al., 2007, Leinhase et al., 2006). However, since the treatment in these studies was an inhibitor of factor B, an early component of the alternative complement cascade, these results likely reflect consequences of generally suppressing the alternative cascade, including terminal MAC formation, as opposed to the effects of suppressing C5a *per se*. In fact, astrocyte-specific overexpression of C5a does not exacerbate pathology in the experimental autoimmune encephalitis (EAE) model of multiple sclerosis (Reiman et al., 2005). Additionally, C5a has been shown to increase expression of glutamate transporter GLT-1 in microglia (Persson et al., 2009) and GluR2 in neurons (Mukherjee et al., 2008). C5a can also protect neurons against glutamate-mediated excitotoxicity and neuronal apoptosis (Mukherjee et al., 2008, Osaka et al., 1999, Mukherjee and Pasinetti, 2001), possibly via modulation of glutamate transporters on glial cells and neurons. C5aR signaling in T

cells has been shown to suppress programmed cell death through a cascade that involves up-regulation of anti-apoptotic Bcl-2 and down-regulation of pro-apoptotic Fas (Lalli et al., 2008). This suggests that another mechanism by which C5a could mediate neuroprotection is modulation of survival signals, in cells within the brain, immune or otherwise.

Contrary lines of evidence have developed around the issue of C5aR signaling in AD brain. A recent study suggests a deleterious role for C5aR1 signaling in Arctic AD mice, whereby deficiency of C5aR1 resulted in restoration of cognitive function and reductions of neuropathologies associated with an AD transgene (Hernandez et al., 2017a). Findings from the same group further suggest that C5aR1 signaling enhances toxicity of fibrillar Aβ in neurons (Hernandez et al., 2017b) and that microglial C5aR expression associates with Aβ deposition and glial recruitment in transgenic AD mice (Ager et al., 2010). Others have also demonstrated a pathogenic role for C5a signaling in brains of mice with experimentally-induced CNS lupus (Jacob et al., 2010), findings that have been subsequently ascribed, at least partially, to pro-apoptotic effects in brain vascular endothelial cells (Mahajan et al., 2016) and disruption of blood-brain barrier integrity (Mahajan et al., 2015).

Other groups, however, have found that mice deficient for C5aR are cognitively impaired, concurrent with down-regulated CREB/CEBP signaling in brain (Gong et al., 2013). Furthermore, restoration of normal brain levels of C5a in AD mice augmented synaptic long-term potentiation and restored cognitive function through C5a-mediated induction of CREB/CEBP signaling (Gong et al., 2013, Gong et al., 2014), suggesting positive effects of C5a on synaptic plasticity. C5a may also protect neurons from Aβ-mediated toxicity (O'Barr et al., 2001), contrary to findings mentioned above. How these C5a-mediated neuroprotective effects are mediated is not fully understood. Studies have shown that exposure of astrocytes and neuronal cells to C5a increased expression of nerve growth factor (NGF), effects that were augmented by co-stimulation with IL-1β (Jauneau et al., 2006). C5a also has a role in stimulating secretion of NGF from dental pulp fibroblasts and may be involved in neurite outgrowth (Chmilewsky et al., 2016). In tubule epithelial kidney cells, C5a has been shown to stimulate secretion of transforming growth factor β (TGF-β), through PI3K/Akt signaling (Yiu et al., 2017). Others have found that C5aR signaling in neurons and glia is associated with anti-inflammatory effects (Gavrilyuk et al.,

2005) and it has been found that C5a promotes proliferation, migration and angiogenic vessel formation by endothelial cells (Kurihara et al., 2010).

In addition, it is well known that C5a increases intracellular calcium concentration in neutrophils (Fujita et al., 2004), macrophages (Roach et al., 2008) and microglia (Moller et al., 1997, Hoffmann et al., 2003), an action that is important for microglial proliferation, migration, ramification, release of cytokines and brain-derived growth factor (BDNF) (Kettenmann et al., 2011). Elevated C5a in the brain of C5a-GFAP transgenic mice may prime microglia, such that these cells respond more rapidly and efficiently to damage caused by repetitive mild TBI in our experiments. This may also explain the elevated levels of Iba1 and Iba1+ cells in our sham C5a-GFAP mice, compared to other groups.

Following TBI, it is likely that C5a is involved with exacerbation of secondary brain injury, as a result of chronic neuroinflammation. However, C5a may also support neuroprotection at certain time points post-TBI. This could occur via multiple possible mechanisms, including modulation of glutamate homeostasis, anti-apoptotic and pro-survival effects, enhanced efficiency of debris clearance and enhanced secretion of growth factors.

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